

## Chapter 18

### Utilization of secondary raw material from fish processing industry

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#### Introduction

Fish and shellfish constitute an important component of global nutrition. Fish protein is an essential source of nutrients for many people, especially in developing countries. Health benefits of fish oil in preventing heart attack and other cardiovascular diseases are well appreciated. Mounting evidences suggest that the benefits of fish consumption are not limited to the well-known effects of fish oil alone. Fish is also a rich source of protein containing all essential amino acids, which is required for the body maintenance and muscular build-up. The protein content of most of the raw finfish meat is in the range of 17 to 22% (g per 100 g), while the cooked portions of some fish such as tuna may have as high as 30% protein. The amino acid score of fish protein compares well with that of whole egg protein, which is considered as a standard protein source. Fish is also rich in the non-protein amino acid - taurine, which has a unique role in neurotransmission. Apart from nutritional properties, fish proteins also possess a number of functional properties such as emulsifying, foaming, gel forming, water binding and fat binding properties, which are important in product formulations. These functional properties are mainly attributed to the major myofibrillar proteins, actin and myosin.

During the processing of fish generally only the fillets are retained while the bulk of product (up to 66%) is discarded. About 30% of the total fish weight remains as waste in the form of skins and bones during preparation of fish fillets. This waste is an excellent raw material for the preparation of high value products including protein foods. The utilization of fish wastes help to eliminate harmful environmental aspects and improve quality in fish processing. Skin and bone are sources of high collagen content. The average quantity of waste generated during fish and shellfish processing operations (based on average annual marine landing data) is indicated in Table 1.

**Table 1: Waste generation in industrial fish processing in India**

Products	Waste generated (%)
Shrimp products (PD, PUD, HL, etc.)	50
Fish fillets	70
Fish steaks	30
Whole and gutted fish	10
Cuttlefish rings	50
Cuttlefish whole	30
Cuttlefish fillets	50
Squid whole cleaned	20
Squid tubes	50
Squid rings	55

Source: Anon (2005)

At present, India is the second largest producer of fish in the world with second position in aquaculture production as well as in inland capture fisheries. The total fish production during 2013-14 (provisional) is registered at 9.58 mMT, with a contribution of 6.14 mMT from inland sector and 3.44 mMT from marine sector (Hand book on fisheries statistics, 2014). This indicates, a minimum of 4MT of fishery waste has been generated every year, even though it is scattered in the domestic and industrial sector.

An important waste reduction strategy for the industry is the recovery of marketable byproducts from fish wastes. Hydrolyzed fish wastes can be used for fish or pig meal as well as fertilizer components. The three most common methods for utilization of aquatic waste (either from aquaculture or wild stock) are the manufacture of fishmeal /oil, the production of silage and the use of waste in the manufacture of organic fertilizer . The utilization of by-products is an important cleaner production opportunity for the industry, as it can potentially generate additional revenue as well as reduce disposal costs for these materials. The transportation of fish residues and offal without the use of water is an important factor for the effective collection and utilization of these by-products. Some viable options for generating wealth from waste are detailed below.

**Fish meal:** Fish meal is highly concentrated nutritious feed supplement consisting of high quality protein, minerals, vitamins of B group and other vitamins and other unknown growth factors. Fishmeal is rich in essential amino acids. It is produced by cooking, pressing, drying and grinding the fish, by-catch fish, and miscellaneous fish. Fishmeal production also

provides a major outlet to recycle trimmings from the food fish processing sector, which might otherwise be dumped at extra cost to the environment and the consumer. Spain, France, Germany, Ireland and the UK produce fishmeal primarily from trimmings. The composition of fishmeal differs considerably due to the variations in the raw material used and the processing methods and conditions. In India, oil sardine (*Sardinella longiceps*) is extensively used for the production of fish meal and oil. Most of the pelagic fishes mentioned earlier are rich in body oil. Hence both fish meal and fish body oil are produced in the same industry. Freshness of fish is very important in getting good quality fish meal. If the fish has lost its freshness, it will have high TVBN content and consequently, the meal produced from it will also contain high TVBN which is unacceptable to shrimp feed industry. In addition to oil sardines, fish dressing waste or cutting wastes (head and viscera) of surimi industry are also used in fish meal manufacture in India. In this case also, the quality parameters regarding freshness of waste have to be maintained. Generally, fish meal produced from fish processing waste, contain low percentage of proteins and high proportion of ash/minerals. Hence, it is not possible to produce Grade I fish meal using only wastes from fish processing industry.

The main objective in the production of fish meal is to reduce the moisture content of fresh fish (70-80%) to about less than 10% in the meal. Oil content in the fish meal should not be more than 10%. Hence, 80 to 90% of oil present in fish has to be removed during fish meal production. The most common methods employed for the manufacture of fish meal are dry rendering and dry rendering process. Dry rendering or dry reduction process is suitable for only lean or non-oil fish such as silver bellies, jew fish, sciaenids, ribbon fish, sole, anchoviella, carcasses of shark, fish offal and filleting waste. In this process, it is dried to moisture content of 10% and pulverized. If the quantity to be handled is sufficiently large a steam jacketed cooker dryer equipped with power devises for stirring is used. Being batch operation the process will have only limited capacity and labour cost is very high. Merit of this process is that the water-soluble materials are retained in the meal. Wet rendering or wet reduction process is normally applied to fatty fish or offal where simultaneous production of fish meal and fish body oil is envisaged. The process consists of grinding, cooking to soften the flesh and bones and to release the oil, pressing to expel the liquor and oil, fluffing the press cake drying, grinding and packing the meal, The press liquor is centrifuged to remove the suspended particles and to separate oil. The stick water is concentrated to retain protein and other valuable components. The press cake is fluffed and dried to a moisture level of 8%.

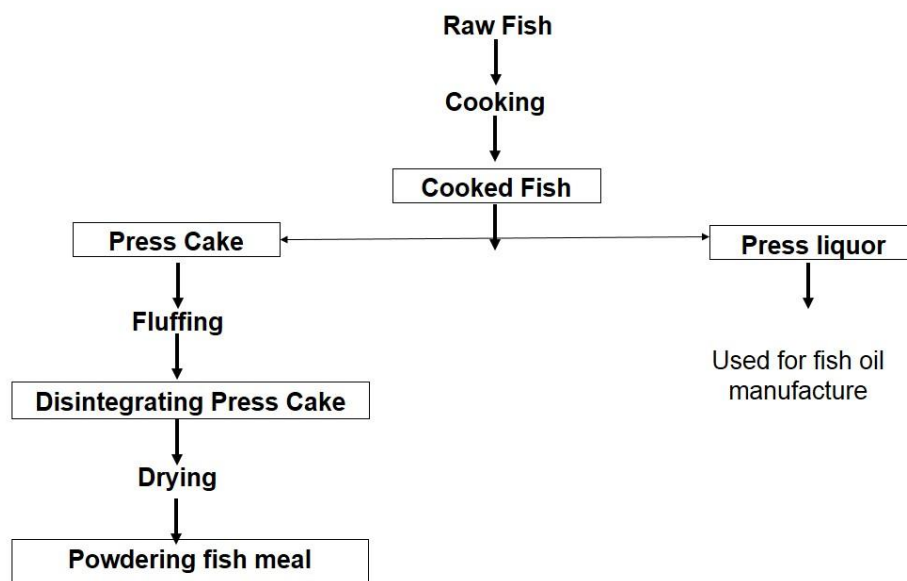


Figure 1. Wet Reduction Method for Fish Meal and Oil Manufacture

### Figure 1. Wet Reduction Method for Fish Meal and Oil Manufacture

Drying is one of the key processes in fish meal production. The dryer used can affect many of the important attributes of fish meal quality. Raw material freshness is important if a producer wants to make premium quality fish meal. Enzymatic and bacteriologic activity in the fish can rapidly decrease the content and quality of the protein and oil. Protein decomposes into amines and ammonia, and both reduce the protein value and recovery of protein. The raw material freshness and drying methods are determining factors of fish meal quality.

BIS has brought out the specification for fish meal as livestock feed for facilitating proper quality control. The proximate composition of fish meal, in general, is protein, 50-60%; fat, 5-10%; ash, 12-35% and moisture, 6-10% employed (Mathew, 2014). Around 15% of the global fish meal demand is met from fisheries resources alone. The projected (2030) annual growth rate in fishmeal use in aquaculture is 1.7%, where the current usage is at a tune of 3.9%. The recent development in captive breeding and rearing high value species such as cobia, grouper, pompano, Nile tilapia, lobster, Asean seabass etc. implies that there is a good scope for flourishing finfish and shellfish production through aquaculture in near future. This inturn highlights the bright future of fish meal industry in coming years, as most of these species demand high protein feeds for their optimum growth.

**Fish protein hydrolysate:** Hydrolysates find application as milk replacer and food flavouring. Enzymes like papain, ficin, trypsin, bromelain and pancreatin are used for hydrolysis. The process consists of chopping, mincing, cooking and cooling to the desired temperature, hydrolysis, sieving, pasteurizing the liquid, concentrating and drying (by vacuum or spray drying). The fish protein hydrolysate have desirable functional properties with potential applications as emulsifiers and binder agents. It can also be used in place of dairy based and plant based protein hydrolysates (Binsi et al., 2016). The peptides formed by the hydrolysis of fish proteins are proven to have bioactive properties like antihypertensive, antithrombotic, immune modulatory and antioxidative properties. Also, they possess superior nutritional and functional properties. A variety of nutraceuticals from FPH are commercially produced and are available in international markets. Oyster peptide extract developed by ICAR-CIFT possess good antioxidant and anti-inflammatory activities. Similarly, hydrolysate made from squilla meat effectively reduced oil absorption in breaded and battered products, when incorporated in the batter mix.

**Fish collagen/gelatin/collagen peptides:** Collagen is the major structural protein in the connective tissue. Collagen extracted from fishes can be used in cosmetics, foods, biomedical applications etc. Gelatin is the partially hydrolysed form of collagen. Both collagen and gelatin are high molecular weight proteins of approximately 300 kDa, hence a considerable proportion is unavailable to human body for biological functions. Consequently, in recent years, much attention has been paid to the development of small molecular weight peptides from the native collagen with improved biological activities. This can be achieved by the process of hydrolysis in which the native collagen/gelatin molecules are cleaved to small fragments of less than 5 kDa. Currently, collagen peptides are being incorporated in a wide array of food products including protein bars, cereal bars, protein drinks, smoothies, yogurts, cold desserts, soups, cured meats etc. Nowadays, collagen/gelatin peptides have gained increasing attention as these peptides exhibit various biological activities such as antioxidant, anti-hypertensive, anti-human immunodeficiency virus, anti-proliferative, anticoagulant, calcium-binding, anti-obesity, anti-diabetic activities and postponement of age-related diseases. ICAR-Central Institute of Fisheries Technology (Cochin, India) has standardised a protocol for the extraction of collagen peptide from fish scale and bone. Further a nutritional mix based on collagen peptides was developed with a protein content of 78%. The product is mainly intended for middle aged and old people, ladies and sports-persons who needs a regular supply of collagen for healthy joints and bones. It may also be beneficial for patients suffering from osteoporosis and long-



term- nursing home residents where there is a possibility of development of pressure ulcers.



**Collagen peptide from fish scale and Nutritional mix formulated by CIFT**

### **Surgical sutures from fresh water fish gut**

Absorbable fine grade sutures are essential for microsurgeries and ophthalmic surgeries. CIFT has developed the method for the preparation of absorbable surgical sutures from fish gut. The production of sutures involves a low cost technology. Fish gut is separated and washed thoroughly to remove impurities and soluble proteins. The collagen fibres separated are twisted, cross-linked and bodied to give fine threads of collages. They are surface smoothened, cut to size and packed in isoporpanol. The packed sutures are sterilized to give absorbable surgical sutures. The sutures developed by this method are evaluated for tenacity, absorbability, freedom from abnormal tissue reaction etc.

**Fish ensilage and foliar spray:** When the animal farms are very near to landing centres it is worthwhile to go for silage production. Fish silage is made from whole fish or parts of the fish to which no other material has been added other than an acid and in which liquefaction of the fish is brought about by enzymes already present in the fish. The product is a stable liquid with a malty odour which has very good storage characteristics and contains all the water present in the original material. Fish silage is preserved against microbial spoilage mainly by the lowered pH, obtained by the added or in-situ produced acid. Specifically, the unionized acid molecules are able to cross the cytoplasmic membrane barrier of the microbial cell while protons ( $H^+$ ) and acid anions cannot. But once inside the cell, the acid mole can ionize and since the membrane traps the ions, the pH gradually comes down killing the cell. Thus it is the unionized acid molecules that are responsible for the preservative action rather than the total acid concentration. At equal concentrations, organic acids are weakly ionized in solution when compared to inorganic acids, thus contain greater amount of unionized

(free acid) molecule making them more effective preservatives. In case of fermented silage, preservation occurs by several means. The presence of fermentable sugar is the beginning of the ensilation process prevents immediate deamination of amino acids by bacteria that would lead to ammonia production and foul smell. Later as the fermentation by lactic acid bacteria becomes dominant, spoilage bacteria are suppressed or killed by the increasing concentration of lactic acid, lowered pH and the production of several antibiotic substances called bacteriocins by the lactic acid bacteria. It is a simple process and it requires little capital equipment particularly if non-oily fish are used. The use of oily fish usually requires oil separation. This involves expensive equipment and is suited to a fairly large-scale operation. The silage may be suitable converted to foliar spray, as foliar feeding is an effective method for correcting soil deficiencies and overcoming the soils inability to transfer nutrients to the plant. The experiments conducted at ICAR-CIFT have shown that foliar feeding can be 8 to 10 times more effective than soil feeding and up to 90 percent of foliar fed nutrients. The application of foliar spray has been advocated in spices like cardamom, black pepper, tea etc and encouraging results have been reported. The quick absorption of the nutrients and precise dosage of foliar sprays has resulted in the success of precision farming of costly vegetables and flowering plants. The controlled nutritional supply through praying is an effective method which gives predicted resulted in most of the cases. The optimized supply of required micro and macro nutrients results in the maximum productivity of the available space and minimizes the wastage of costly inputs.

**Enzymes:** There is a great demand for enzymes with right combination of properties for a number of industrial applications. Enzymes from marine fisheries resources have wide biotechnological potential as they have some unique properties for industrial applications, e.g. in the detergent, food, pharmaceutical, leather and silk industries. Among the enzymes derived from various sources, marine enzymes have certain technological advantages. Some of the distinctive features of enzymes derived from fish include, the higher catalytic efficiency at lower reaction temperatures, and stability at wide range of pH and in the presence of surfactants or oxidizing agents. The higher catalytic activity at lower temperature is a unique property that further permits to process foods at low temperatures such as fruit juices, thereby protects heat-labile food components and reduces the energy cost. Similarly, the lower thermo-stability of marine enzymes would permit their complete inactivation by mild heat treatments, whereas the enzymes from microbial and plant resources often requires heating at above 90°C for a minimum duration of 10 min

for stopping the enzymatic reaction. World-wide the sales of industrial enzymes are growing at a fat rate. Presently, industrial enzymes are mostly derived from microorganisms and to a lesser extent from plant and bovine sources. So far, there is only very limited use of marine derived enzymes by industry. The reason may be the limited basic informations on these enzymes, the seasonal nature of raw material availability, the psychological inertia of the public towards fish offal, and to a greater extent, due to the lack of proper techniques for the recovery of enzymes from fish processing waste which comprises of a complex mixture of various biomolecules such as proteins, lipids, minerals, glyco-proteins etc. It is suggested that future research may be focused on the development efficient and cost-effective technologies for the recovery of various enzymes from fishery resources, so that some of the unique properties of marine enzymes may be exploited in various food applications, and thereby, obtain a share of lucrative industrial enzyme market to increase the profit for fish processing industry.

**Fish calcium:** In marine ecosystem, there is a large amount of calcium, mainly in the form of calcium carbonate and calcium phosphate, distributed as skeletal elements of teleosts, exoskeletal elements of molluscs or as coral deposits. In marine ecosystem, there is a large amount of calcium, mainly in the form of calcium carbonate and calcium phosphate, distributed as skeletal elements of teleosts, exoskeletal elements of molluscs or as coral deposits. The bone fraction, which comprises approximately 15-20% of the total body weight of fish has high calcium content. Calcium and phosphorus comprise about 2% (20 g/kg dry weight) of the whole fish. Generally, fatty fish have lower ash levels compared to lean species. The filleting wastes of tuna and other bigger fishes are very good sources for calcium when the quantity of calcium is concerned. Also, the bone structure differs between species since a large number of teleosts have acellular bone (bone without enclosed osteocytes). Cellular bones are confined to only a few fish groups, e.g. Salmonidae. The higher surface to volume ratio in acellular fish bone is likely to increase the calcium availability compared to cellular bone. The ash content is highest in lean fish species with acellular bones. Apart from that exoskeleton of mollusks and coral deposits are excellent source of calcium. However, the calcium from these deposits are mainly in the form of calcium carbonate. Central Institute of Fisheries Technology, Cochin has optimised the process to extract from fish bone which is mainly treated as processing discards during filleting operation of larger fishes, viz tuna, carps etc. The calcium powder was supplemented with vitamin D which is known to enhance absorption and bioavailability of calcium in the body. *In vivo* studies conducted at CIFT in albino rats have shown that



fish calcium powder supplemented with vitamin D has improved the absorption and bioavailability.

**Chondroitin Sulphate:** Chondroitin sulfate (CS) is a major component of the extracellular matrix (ECM) of many connective tissues, including cartilage, bone, skin, ligaments and tendons. It is formed by repeating disaccharide units of glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc). The skeleton of shark and ray is therefore an attractive source of CS. This polymer is at the moment object of increasing attention in the engineering of biological tissues, especially in connection with the repair of bone, cartilaginous and cutaneous wounds. It is part of a large protein molecule (proteoglycan) that gives cartilage elasticity. Chondroitin sulphate has been used for the treatment of arthritis. Its high content in the aggrecan plays a major role in allowing cartilage to resist pressure stresses during various loading conditions. Chondroitin sulfatation profile has been described in cartilage CS is sold as over the counter dietary supplement in North America and is a prescription drug under the regulation of the European Medicine Agency (EMA) in Europe. The extraction of CS includes the following steps: cartilage hydrolysis (with strong alkalis or using proteases), ethanolic precipitation of the hydrolysates and treatment of the redissolved precipitate (with ionic exchange resins or by means of dialysis) in order to eliminate remaining peptides and salts (Sumi et al., 2002).

**Squalene:** Squalene is a natural dehydrotriterpenic hydrocarbon ( $C_{30}H_{50}$ ) with six double bonds, known as an intermediate in the biosynthesis of phytosterol or cholesterol in plants or animals. It is present in the liver oil of certain species of deep sea sharks mainly *Centrophorus* and *Squalidae* spp. In the case of deep-sea sharks, the liver is the main organ for lipids' storage, being in the same time an energy source and means for adjusting the buoyancy. The liver oil of these species contain high percentage of squalene (90%) which can be isolated and purified and can be used as a dietary supplement. In their case, the unsaponifiable matter represents 50–80% of the liver, the great majority thereof being squalene. Squalene has a melting point lower enough to allow the cooling composition to remain liquid, even at temperatures between  $-10^{\circ}C$  and  $-60^{\circ}C$ , unlike the ordinary oily topical drugs. Squalene is used as a bactericide, an intermediate in the manufacture of pharmaceuticals, organic colouring matter, rubber, chemicals, aromatics, in finishing natural and artificial silk and surface active agents. Nowadays it is extensively used as an additive in pharmaceutical preparations, cosmetics and health foods. Squalene is found to be a proficient chemo preventive agent against lung metastasis in mice bearing lung carcinoma. Squalene revives damaged body cells and aids to revitalize cell generation. Its chief attribute is the

protection of cells from oxidation reactions. Squalene assists to clean, purify, and detoxify the blood from toxins, facilitating systemic circulation. It purifies the gastrointestinal tract and kidneys, causes better bowel movement and urination. Squalene was also used as an adjuvant in vaccines, stimulating the immune response and increasing the patient's response to vaccine. It is added to lipid emulsions as drug carrier in vaccine applications. Squalene helps in regulating the female menstrual cycle and also improves irregular and abnormal cycles. Shark liver oil remains the richest natural source of squalene, even though it is widespread in animal and vegetal kingdom. ICAR-CIFT has standardised the protocol for extracting squalene from shark liver oil.

**Hydroxyapatite (HAp):** Hydroxyapatite is the major mineral component of bone tissue and teeth, with the chemical formula of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . The composition HAp derives from biological sources differs from that of synthetic hydroxyapatite, due to the presence of several ionic substitutions in the lattice, such as  $\text{CO}_3$ , F,  $\text{Mg}^{2+}$  and  $\text{Na}^+$ . It is a member of the calcium phosphate group with 1.67 stoichiometric of Ca/P ratio. It is one of the few materials, classified as a bioactive biomaterial that supports bone in growth and osseointegration when used in orthopedic, dental and maxillofacial applications. Fish bone and scale is a rich source of hydroxyapatite. The hydroxyapatite content of fish skeleton may vary between 40-60%. Generally, very high heat treatment is used for extraction of HAp from bone and this temperature gives a higher strength to HAp structure. The high temperature also burns away any organic molecules such as collagen protein. Hydroxyapatite, found in fish is chemically similar to mineral components of bone and hard tissues in mammals. Approximately, 65-70% of the fish bone is composed of inorganic substances. Almost all these inorganic substances are hydroxyapatite composed of calcium, phosphorous, oxygen and hydrogen.

**Pigments:** Astaxanthin, fucoxanthin, melanin etc. from different fish resources are found to have a variety of bioactive properties. The filleting discards of salmonids and the shell wastes of crustaceans contain significant amounts of carotenoid pigments such as astaxanthin and canthaxanthin. Normally, wild caught shrimps will have more pigmentation compared to their cultured counterparts. For eg. level of astaxanthin in wild caught *P. monodon* is reported as 55 mg /kg as compared to 18mg/kg for cultured variety. The carotenoid content in normal individual of *P. monodon* is reported very high (78-80%) as compared to their blue varieties (7-8%). Total carotenoid content varies between species and body components. Highest carotenoid content was reported in the head of deep-sea shrimp (*A. alcocki* 180-185  $\mu\text{g/g}$ ) and marine shrimp (*P. styliifera* 150-155  $\mu\text{g/g}$ ) followed by *P. monodon* (120-

135µg/g), *P vannamei* (120-130µg/g). High levels of carotenoids were also reported in carapace of *A alcocki* (115-120 µg/g), *S indica* (117-120.0 µg/g) and *P stylifera* (100-105µg/g). Relatively low levels of carotenoids were reported in shrimp *P indicus* and fresh water prawn *M. rosenbergii* and crabs. The protective role of carotenoids against the oxidative modification of LDL cholesterol could be explored by incorporating in health drinks. Carotenoids are also highly sought after as natural food colours. Cephalopod ink is another less-tapped reservoir of a range of bioactives having therapeutic and curative values. It is an intermixture of black pigment melanin, glycosaminoglycans, proteins, lipids, and various minerals. Cephalopod ink has been reported to have anti-radiation activity, antitumor activity, immunomodulatory activity, procoagulant function and so on. The pigment melanin can be used both as a natural colorant as well as antioxidant, in addition to a number of other therapeutic and prophylactic properties including anticancer, antihypertensive, Anti IDA etc.

### **Pearl Essence**

Pearl essence is the suspension of crystalline guanine in a solvent,. It is the iridescent substance located in the epidermal layer of the scales of the pelagic fish. This is used for coating the objects to give them a lustrous effect. The scales are placed in 10-15% brined solution and the brine is later drained and scaled squeezed and compressed. Pearl essence is extracted by washing and scrubbing the guanine from the scales. Centrifugation is carried out for separating the pearl essence from wash liquid. For purification of guanine, the protein concentrate is digested with pepsin in acid at 25- 30 °C for 50 hours. Fat is removed with benzene or ether. Finally guanine is removed by centrifugation and suspended in water or in non aqueous liquid.

### **Fish glue**

Fish glue is made from fish skins (the better quality glue) and of fish heads (the lesser quality glues). Skin can be salted for shorter period of storage but can be dried for longer period of storage. For extracting glue .for extracting glue from fish skin, the skins are initially cooled and the chloride is removed to less than 0.1% by washing. For 1-2 hours if fresh and 12 hours if they are stored) in cold running water in a roller mill. After the water treatment, the skins are placed in 0.2% NaOH or CaO, neutralized with 0.2% HCl, and again rinsed in running cold water. The skins now swollen are mixed with an equal weight of water and steam is added. Addition of 1.9 litre of glacial acetic acid during heating will make the final glue a clearer product. First cooking is for eight hours and the glue layer is strained. Subsequent cooking will give a weaker glue.

## **Fish maws and Isinglass**

The word isinglass is derived from the Dutch and German words which have the meaning sturgeon's air bladder or swimming bladders. Not all fish air bladder are suitable for isinglass production. The air bladder of deep water hake is the most suitable for production of isinglass. In India air bladders of eel and cat fishes are used for the production of isinglass.

The air bladders are separated from the fish, and temporarily preserved in salt during transport. On reaching the shore, they are split open, thoroughly washed and the outer membrane is removed by scraping and then air dried.

The cleaned, desalted, air dried and hardened swimming bladders (fish maws) are softened by immersing in chilled water for several hours. They are mechanically cut into small pieces and rolled or compressed between hollow iron rollers that are cooled by water and provided with a scraper for the removal of any adhering dried material. The rolling process converts the isinglass into thin strips or sheets of 1/8 to 1/4 “; thickness. There are processes for the production of isinglass in powder form.

Isinglass dissolves readily in most dilute acids or alkalis, but is insoluble in alcohol. In hot water isinglass swells uniformly producing opalescent jelly with fibrous structure in contrast to gelatin. It is used as a clarifying agent for beverages like wine, beer, vinegar etc. by enmeshing the suspended impurities in the fibrous structure of the swollen isinglass.

India exports dried fish maws, which form the raw material for the production of isinglass and such other products. Process has been developed to produce the finished products from fish maws.

## **Further reading**

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